

Application No.: 09/973,089
Amendment Dated 1 April 2005
Filed with RCE in reply to Advisory Action of 1 March 2005

REMARKS

Claim 1 has been amended to limit the differentiation agent to abscisic acid (ABA).

In view of the amendment to claim 1, claims 5-8, 10-11, 14-17, 19-20, 29 and 38 have been canceled.

It is submitted that this amendment does not constitute new matter and its entry is requested.

The Examiner has rejected claims 1-3, 5-8, 10, 11, 14-17, 19, 20 and 22-43 under 35 U.S.C. §103(a) as being obvious over Wenck et al. (*Plant Molecular Biology* 39:407-416, 1999) taken with Rutter et al. (US 5,731,204) and Levee et al. (*Molecular Breeding* 5:429-440, 1999). The Examiner stated that this rejection is maintained for the reasons of record set forth in the Office Action mailed 9 April 2004. It is submitted that the claimed invention is not obvious from the teachings of the cited art.

In accordance with the claimed invention, genetically modified plants of pine of the subgenus *Pinus* are regenerated by selecting transgenic embryogenic pine cells using a selection medium comprising a selection agent and a differentiation agent and then regenerating plants from the selected transgenic embryogenic pine cells. The pines of the subgenus *Pinus* are selected from the group consisting of Southern yellow pines and hybrids thereof. The differentiation agent is abscisic acid (ABA). Applicants discovered that the presence of the specified differentiation agent in the selection medium allowed for the first time the regeneration of transgenic plants of pine of the subgenus *Pinus*, particularly Southern yellow pines and more particularly lines of certain elite genetic backgrounds of the Southern yellow pines. These pines are hard pines.

At page 3 of the Office Action of 9 April 2004, the Examiner stated,

Wenck et al. teach a method of genetically transforming Norway spruce using *Agrobacterium* and selecting the transformed embryogenic lines using kanamycin as an agent to select transformants and incorporating into the media abscisic acid which acts as an agent to regulate differentiation of embryos from embryogenic cells (page 409, left column, first line and right column, ‘Embryo maturation’ paragraph, respectively.)

However, as Applicants detailed in their previous Amendments, Wenck et al. does not contain any such disclosure. Wenck et al. does disclose the use of a selection agent, i.e., kanamycin,

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in the selection medium. The selection medium is 471 medium. This medium does not contain ABA as shown by the medium components in Table 2 on page 410. Thus, transformed cells are selected on a medium that does not contain ABA. “Selected” cell lines are then cultured to produce mature embryos. The culturing of the “selected” cell lines is done on medium that contains ABA. It is the production of mature embryos from selected cells that is done on medium containing ABA not the selection of transgenic embryogenic cells on a selection medium that contains ABA. Thus, Wenck et al. does not disclose the use of the claimed differentiation agent in the selection medium.

Furthermore, Wenck et al. does not describe the selection and regeneration of genetically modified pine of the genus *Pinus* subgenus *Pinus*. It merely discloses the selection and regeneration of genetically modified Norway spruce (*Picea abies*) which is not a pine. In fact, Wenck et al. teaches that it was unable to transform and regenerate genetically modified Southern yellow pine plants. *See*, page 413, left column.

Consequently, not only does Wenck et al. not teach selecting and regenerating genetically modified Southern yellow pine plants, but it also does not teach using ABA in the selection medium.

As noted, Wenck et al. only teaches the transformation and regeneration of Norway spruce, which is not a pine, let alone a pine of the subgenus *Pinus*. Furthermore, Wenck et al. teaches that it was unable to transform and regenerate genetically modified Southern yellow pine plants using the same procedure as used for Norway spruce, i.e., using a selection medium that included a gelling agent in an amount of 0.2%, which itself is not encompassed by the claims. *See*, page 413, left column. Thus, Wenck et al. does not teach either (a) the successful selection and regeneration of genetically modified hard pines, i.e., pines of the genus *Pinus*, subgenus *Pinus* in which the hard pines are selected from the group consisting of Southern yellow pines and hybrids thereof or (b) the use of the claimed differentiation agent in the selection medium.

In summary, Wenck et al. does not teach the selection and regeneration of genetically modified pines, let alone hard pines. In addition, Wenck et al. does not teach the use of the claimed differentiation agent in the selection medium for the selection and regeneration of genetically

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modified hard pines. Specifically, Wenck et al. does not teach the use of a differentiation agent in selection medium in which the differentiation agent is ABA.

At page 7 of the Office Action of 9 April 2004, the Examiner stated,

The Office contends, that absent evidence to the contrary, the method of Wenck et al. of transforming and regenerating one conifer, Norway spruce, can be applied to transforming and regenerating another conifer, that being Southern yellow pines.

As Applicants have previously detailed, Wenck et al., itself contains evidence to the contrary and clearly demonstrates that the method of Wenck et al. useful for one conifer species, i.e., Norway spruce, cannot be applied to another conifer species, specifically a Southern yellow pine. Wenck et al. discloses experiments for the transformation and regeneration of not only Norway spruce, but also loblolly pine, a Southern yellow pine. Wenck et al. report the successful regeneration of transformed cells of Norway spruce. However, Wenck et al. report the **unsuccessful** regeneration of transformed loblolly pine. *See* page 413, left column. Thus, the very art cited by the Examiner contains the very evidence that the method of Wenck et al. for transforming and regenerating transformed plants of one conifer is not applicable to transforming and regenerating another conifer. The Examiner has not provided any rebuttal of, or evidence to rebut, this specific teaching of the cited Wenck et al. Thus, Applicants have provided evidence to the contrary to what the Examiner contends can be taken from the cited prior art. In view of this contrary evidence, it is submitted that the presently claimed invention is not obvious from the cited prior art.

In addition, Applicants have previously also demonstrated that none of the secondary references disclose the use of a differentiation agent as specified in the claims in a selection medium. There is no disclosure of transgenic embryogenic pine cells of Southern yellow pines in Rutter et al., and consequently, there is no disclosure of a selection medium. Since there is no disclosure of a selection medium in Rutter et al., there is no disclosure of the use of the differentiation agent specified in the claims, i.e., ABA, in a selection medium. Thus, Rutter et al. does not disclose these elements of the claimed invention. Similarly, Levee et al. does not disclose the use of the differentiation agent specified in the claims, i.e., ABA, in a selection medium for selecting transgenic

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tissue. Furthermore, Levee et al., which relates to a soft pine (i.e., a pine of the subgenus *Strobus*), does not describe the regeneration of genetically modified pine plants of the subgenus *Pinus*. As shown in the paper attached to the Amendment After Final filed on 18 January 2005, the subgenus *Pinus* are hard pines, whereas the subgenus *Strobus* are soft pines. Thus, Levee et al. does not disclose these elements of the claimed invention. Furthermore, in view of the evidence to the contrary provided by Applicants, it is submitted that the method of Levee et al. cannot be applied to Southern yellow pines. Since none of the cited references disclose the use of the differentiation agents specified in the claims in a selection medium and do not disclose the regeneration of genetically modified Southern yellow pines and hybrids thereof, the cited references do not and cannot render the claimed invention obvious. On this basis alone, Applicants submit that the Examiner should withdraw the obviousness rejection.

Further in the Amendment filed 9 August 2004, Applicants described the well known differences between hard and soft pines and the prior inability to recover selected cells at a level which would be commercially useful for producing transgenic hard pine, especially for Southern yellow pines and hybrids thereof, all of which are hard pines. Applicants supported their position by the Rule 132 Declarations of Dr. Connett-Porceddu, Dr. Becwar, Dr. Canavera and Dr. Mann. The Examiner has summarized Applicants arguments and the Rule 132 Declarations at pages 3-4 of the present Office Action.

In response to these arguments, the Examiner stated on page 4 of the most recent Office Action that “it is noted that features upon which applicant relies (i.e., a method for regenerating genetically modified plants of pine of the genus *Pinus* subgenus *Pinus*, and the use of ABA in the selection medium) are not recited in the rejected claim(s).” The Examiner contends that because “soft and hard pines can interbreed if correct timing and other conditions are provided,” then the language “hybrids thereof” encompasses soft pines which are regenerable using the method of Wenck et al. To support his statement concerning the interbreeding of soft and hard pines, the Examiner cites to the Rule 132 Declaration of Dr. Connett-Porceddu, page 3, number 9. This

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particular Declaration is Exhibit 2 to the Rule 132 Declaration of Dr. Connett-Porceddu filed for the present application.

However, the Examiner is incorrect in his analysis of the statement in Dr. Connett-Porceddu's Declaration. In her Declaration, Dr. Connett-Porceddu stated,

As is commonly known, **hard pines are unable to breed with soft pines, though they can interbreed readily**, if the correct timing and other conditions are provided, **with other hard pine species** (a seminal reference is Critchfield and Little, 1966, *Geographic distribution of the pines of the world*, USDA Forest Service Miscellaneous Publication 991, Washington, D.C. (copy attached as Exhibit 2); see also Little and Critchfield, 1969, *Subdivision of the genus Pinus (Pines)*, USDA Forest Service Miscellaneous Publication 1144, Washington, D.C. (copy attached as Exhibit 3)). (emphasis added)

It is submitted that the Examiner has misread this passage from Dr. Connett-Porceddu's Declaration as he stated in the most recent Office Action.

Instead of stating that soft pines and hard pines can interbreed, Dr. Connett-Porceddu specifically states that "hard pines are unable to interbreed with soft pines." Dr. Connett-Porceddu further specifically states that "they can interbreed readily ... with other hard pines." The subject in the quoted passage is hard pines. Thus, the "**they**" in the quoted passage refers to "**hard pines**" and not soft pines as the Examiner has apparently interpreted this passage. The interbreeding of hard pines with other hard pines can occur "if the correct timing and other conditions are provided." Thus, it is the hard pines that interbreed with other hard pines, whereas hard pines and soft pines are unable to interbreed. No art has been cited by the Examiner that would contradict the statements of Dr. Connett-Porceddu. Consequently, because hard pines and soft pines cannot interbreed, it is submitted that hybrids of Southern yellow pines (hard pines) are only hybrids of hard pines and thus, the claims do not encompass soft pines.

In addition, as previously noted, Wenck et al. does not describe a method for the selection and regeneration of pine of the subgenus *Pinus*. It only shows the selection and regeneration of Norway spruce. There is no disclosure or suggestion in Wenck et al. that its method could be applied to any pine species of any *Pinus* subgenus, let alone Southern yellow pines and hybrids thereof. In

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fact, Wenck et al. shows that the method does not work for selecting and regenerating pine of the subgenus *Pinus*, and specifically shows that the method does not work for a Southern yellow pine. Also as previously noted, Wenck et al. does not describe or suggest the specified differentiation agent, ABA. Thus, Wenck et al. does not describe the selection and regeneration of genetically modified plants of Southern yellow pines and hybrids thereof and does not describe the claimed differentiation agent.

Thus, it is submitted that the features upon which Applicants rely are set forth in the claims, namely, (a) a method for regenerating genetically modified plants of pine of the genus *Pinus* subgenus *Pinus* in which the pines are selected from the group consisting of Southern yellow pines and hybrids thereof, and (b) the use of a differentiation agent in the selection medium in which the differentiation agent is ABA. The features of the claimed invention are not disclosed or suggested in any of the art cited by the Examiner. Thus, Applicants submit that the claimed invention is not obvious from the cited art.

As detailed in the Amendment filed 9 August 2004 and supported by the Rule 132 Declaration of Dr. Connett-Porceddu, one feature of the invention which enabled the recovery of transgenic cells for regenerating transgenic plants was the use of a differentiation agent in the selection medium. The use of the claimed differentiation agent was in addition to the selection agent which is used to select transgenic cells. The use of the differentiation agent ensured that transgenic cells could be recovered following selection and these transgenic cells could be regenerated into transgenic plants in commercially useful quantities. For example, prior art techniques resulted in a 26% rate of regeneration for loblolly pine (*Pinus taeda*), whereas the present invention resulted in a 71% rate of regeneration. Similarly, for elite families, the prior art was unable to achieve regeneration, whereas the present invention achieved an 80% rate of regeneration. This result of the present invention is not predictable from the art cited by the Examiner and from the knowledge in the art as demonstrated by the above noted Rule 132 Declarations.

Applicants submit that the Amendment filed on 9 August 2004, in combination with the Rule 132 Declarations filed therewith, clearly demonstrated that there are, and were at the time of the

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present invention, well known differences between hard and soft pines. That Amendment and the Rule 132 Declarations further demonstrated the unobvious nature of plant transformation and regeneration in these species. These differences (i.e., the differences between hard and soft pines and the differences in plant transformation and regeneration in hard pines and soft pines) present additional evidence (in addition to that provided by Wenck et al.) that transformation and regeneration in one conifer species could not be applied to another conifer species. The comments and evidence demonstrating the unobvious nature of the present invention are incorporated herein by reference. The Examiner has not cited any evidence that would rebut the evidence previously presented.

For example, Levee et al., the only cited prior art that discloses regeneration of a transformed pine, discloses the transformation and regeneration of pine of the subgenus *Strobus* which, according to this reference, “is the first work on genetic transformation on **this pine species** as well as the first report of successful stable genetic transformation of **a pine species** using a disarmed strain of *A. tumefaciens*”. (See page 36, first paragraph of Discussion, emphasis added). Levee et al. does not disclose the transformation and regeneration of pine of the subgenus *Pinus*. The claims of the present application are clearly directed to pine cells of the *Pinus* subgenus. It is well known to those skilled in the art that somatic embryogenesis systems for soft pines are different from those for hard pines, such as Southern yellow pines and hybrids thereof. It is not insignificant that Levee et al. utilized a soft pine which is more easily regenerated than hard pines. Although the Examiner cited art showing transformation and regeneration of soft pine, he has not cited any art showing transformation and regeneration of hard pines as set forth in the claims. In addition, the art that the Examiner cited, i.e., Wenck et al., specifically shows that hard pines had not been transformed and regenerated by a method that was successful for one conifer species.

Furthermore, it is submitted that there have been no reports in the literature of the regeneration of plants following stable transformation of embryogenic cultures of any pines of the *Pinus* subgenus by *Agrobacterium*. These facts are all supported by the Rule 132 Declarations that were previously filed. For example, it is Dr. Connett-Pordeddu’s opinion that a skilled artisan would

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have no expectation of success for modifying the method of Wenck et al. to regenerate genetically modified plants of Southern yellow pines. *See Paragraph 12 of the Connell Declaration.* In addition, Dr. Connell-Porceddu also states that a skilled artisan would not expect that the method of Levee et al. for soft pines could be used or routinely modified for use with hard pines. *See Paragraph 13 of the Connell Declaration.* Thus, Dr. Connell-Porceddu concludes that a skilled artisan would have no expectation of success for modifying the method of Wenck et al. to regenerate genetically modified plants of Southern yellow pines or hybrids thereof. *See Paragraphs 15-17 of the Connell Declaration.* The Examiner has not cited any art that contradicts the evidence and opinions set forth in Dr. Connell-Porceddu's Declaration.

As stated in the Amendment filed 9 August 2004, it is submitted that the previously filed Rule 132 Declarations establish that the present invention is not obvious from the teachings of Wenck et al., Rutter et al. and Levee et al. It is further submitted that the use of a differentiation agent as specified in the claims in a selection medium also containing a selection agent to select transgenic embryogenic pine cells of Southern yellow pines and hybrids thereof is not obvious from the teachings of Wenck et al., Rutter et al. and Levee et al. Thus, it is submitted that the claimed invention is not obvious from the teachings of Wenck et al., Rutter et al. and Levee et al. Withdrawal of this rejection is requested.

In summary, none of the cited references disclose the use of a differentiation agent that is ABA in a selection medium which is used for selecting transgenic embryogenic pine cells of the genus *Pinus* subgenus *Pinus* selected from the group consisting of Southern yellow pines and hybrids thereof. Also, none of the cited references disclose the regeneration of genetically modified plants of the genus *Pinus* subgenus *Pinus* selected from the group consisting of Southern yellow pines and hybrids thereof. Because hard pines, which include Southern yellow pines, are unable to breed with soft pines, the claims do not encompass soft pines, i.e., hybrids of Southern yellow pines do not encompass soft pines. Furthermore, the evidence presented in the prior art and in the Rule 132 Declarations demonstrate that there is no expectation of success in this art, and more particularly, that there is no expectation that methods useful for one conifer species, including soft pines, can be

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used for another conifer species, including hard pines such as Southern yellow pines and hybrids thereof. Thus, it is submitted that the combination of Wenck et al., Levee et al. and Rutter et al. does not render the claimed invention obvious. Withdrawal of this rejection is requested.

In view of the above amendments and remarks, and in conjunction with the remarks made in the previous amendments and the previously filed Rule 132 Declarations, it is believed that the claims satisfy the requirements of the patent statutes and are patentable over the prior art. Reconsideration of the instant application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

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